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Synthesis and *in vitro* Evaluation of Fluorinated Derivatives of HU210 and HU211 as Novel Candidate PET Tracers

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Abstract

Introduction

CB1 receptors are involved in psychiatric, neurological, and behavioral disorders, as well as in non-CNS pathologies such as liver fibrosis, metabolic disorders such as diabetes, and cancer. PET imaging has emerged as a powerful technology for the diagnosis and staging of CNS and non-CNS pathologies, as well as a tool in drug design and development. We and others have actively investigated novel CB1-targeted PET tracers, however few [¹⁸F]-tracers have demonstrated true potential to image CB1 receptors *in vivo*. Recently, Kassiou et al. have shown that synthetic cannabinoid ligands carrying a terminal fluorine atom on linear aliphatic chains have superior or equivalent pharmacological properties relative to non-fluorinated counter-parts. This observation supported our idea to investigate novel selectively fluorinated cannabinoid analogues as candidate PET tracers, in particular HU-210 (Fig. 1) which is a potent (–)-1,1-dimethylheptyl analogue of 11-hydroxy- Δ^8 -tetrahydrocannabinol. This cannabinoid receptor agonist was reported to have 0.061 and 0.52 nM K_i values at cloned human CB₁ and CB₂ receptors, respectively. HU-210 also displays agonist activity at GPR55 (EC_{50} = 26 nM). Interestingly, its enantiomer HU-211 (dexanabinol) has very different pharmacological properties, being essentially inactive as a cannabinoid but active as NMDA receptor ligand (IC_{50} = 11 μ M for inhibition of [³H]MK-801 binding to rat forebrain membranes). HU-211 has been shown to have neuroprotective effects and is being clinically tested for treating traumatic and ischemic brain injury. HU-211 is also an effective free radical scavenger, and is currently in phase-II clinical trials for the therapy of solid tumours. Despite the strong interest in HU-210 and HU-211, to our knowledge the use of radiolabelled analogues for *in vivo* imaging has never been reported, and fluorinated analogues have not been described either. We hereby report a synthesis of terminally fluorinated HU-210 and HU-211 analogues, and demonstrate that both compounds maintain their native pharmacological properties *in vitro*, thus supporting the hypothesis that [¹⁸F]-labelled HU210 and HU-211 could be valuable PET tracers for *in vivo* imaging.

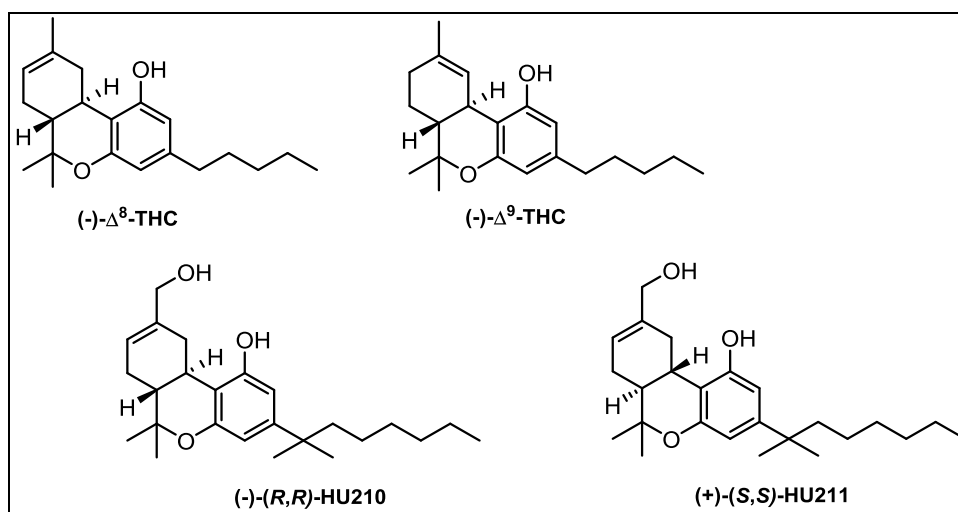


Figure 1.

Results

The synthesis of the two stereochemically pure fluorinated compounds (-)-(R,R)-HU210F and (+)-(S,S)-HU211F (Fig. 2), which are enantiomers, was undertaken.

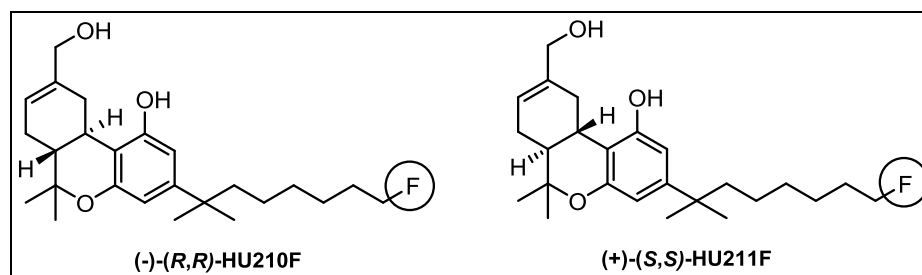
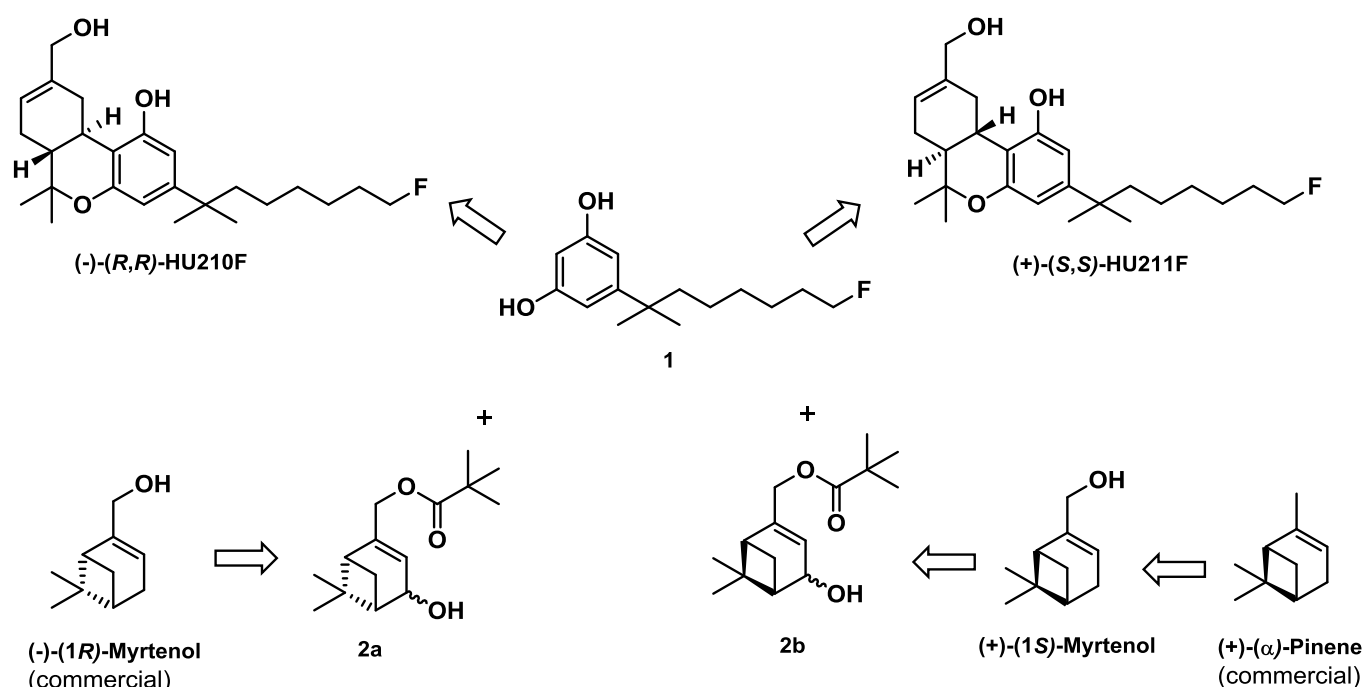


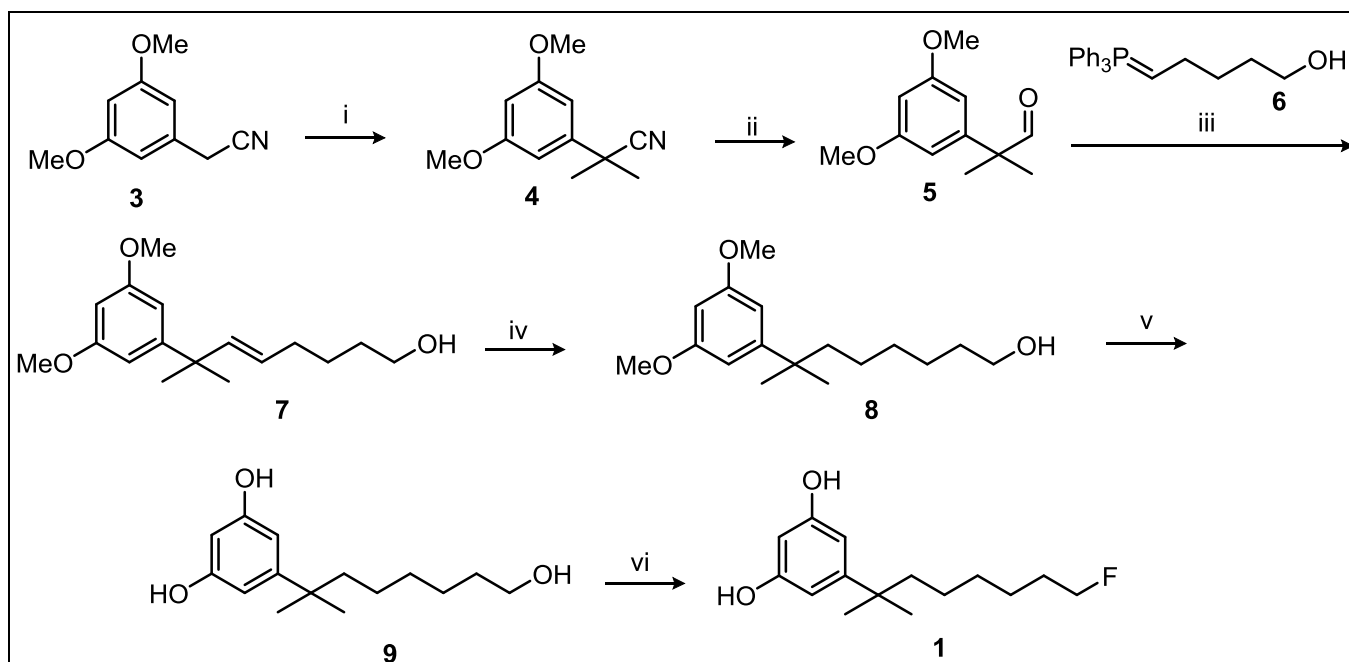
Figure 2.

The retrosynthetic approach to the target compounds HU-210F and HU-211F (**Scheme 1**) was designed taking advantage of the chemistry published by Mechoulam¹ and Huffman². Fluorinated resorcinol **1** was identified as the key intermediate to be reacted with both enantiomers of 4-hydroxy-myrtanyl pivalate **2a,b** forming the desired THC-like structural frameworks.



Scheme 1. Retrosynthetic approach to (-)-(R,R)-HU210F and (+)-(S,S)-HU211F

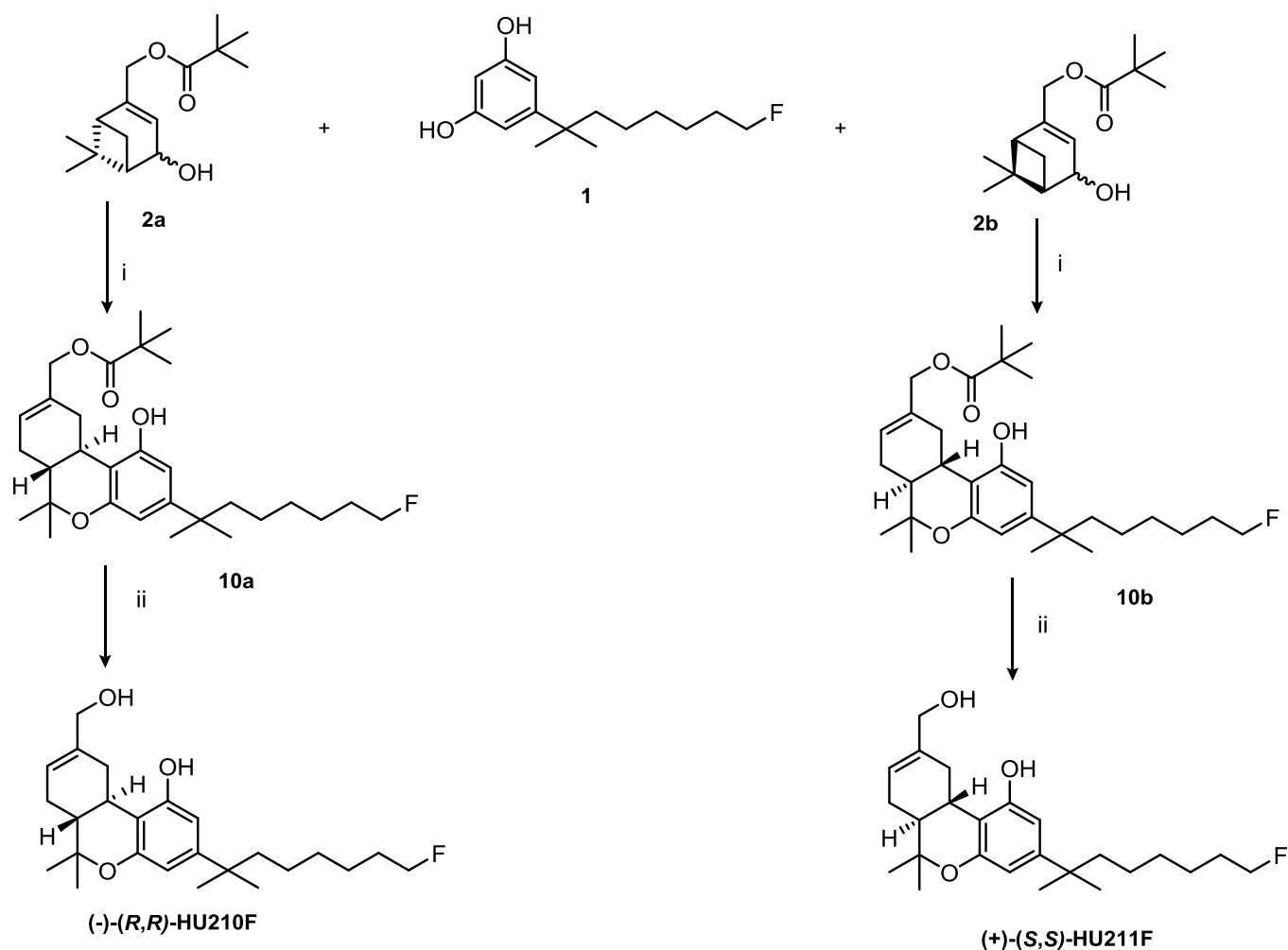
Synthesis of the key fluorinated building block **1** proved to be challenging. In fact, a number of unsuccessful approaches to **1** were explored (more information is provided in the Electronic Supporting Information section) before identifying a successful synthetic strategy (**Scheme 2**). The synthesis of **1** started with the alkylation³ of commercially available dimethoxyphenyl)acetonitrile (**3**), that afforded the α,α -dimethylnitrile **4** in excellent yield. Subsequent hydride reduction⁴ afforded the aldehyde **5** that yielded the unsaturated alcohol **7** by means of a Wittig olefination⁴ with freshly synthesised⁵ phosphine **6**. The alkene bond was then hydrogenated (Pd/C, H₂, in EtOAc)⁴ to give compound **8** in quantitative yield. During the first deprotection's attempt (BBr₃ in DCM from -78 °C to -10 °C)⁶ the undesired nucleophilic substitution of the primary OH of **8** with a Br occurs, leading to the corresponding unwanted alkyl bromide. However, treatment of **8** with MeMgI at 170 °C⁷ provided the deprotected alcohol **9** in very good yield. Next, **9** was dehydroxyfluorinated with DAST⁸ (the use of DEOXOFLUOR provided lower yields) affording the target compound **1**.



Scheme 2. Synthesis of common intermediate **1**

Reagents and conditions: i) NaH, MeI, DMF, 0°C to rt, 2h (98%); ii) DIBALH, DCM, rt, 1h (93%); iii) **6**, LiHMDS, THF, 0°C to rt, on (92%); iv) H₂ 1 atm, 10% Pd/C, EtOAc, on (quantitative); v) MeMgI, Et₂O/THF, 0°C to 170°C, 1h (70%); vi) DAST, DCM, -78°C, 0°C, 15 min (50%).

The synthesis of (-)-(R,R)-HU210F was achieved from commercially available (-)-(1*R*)-Myrtenol (≥95% ee) that was transformed into 4-hydroxy-myrtanyl pivalate **2a** (Scheme 3) using the method of Huffman (Ref. 1 and 2). The synthesis of (+)-(S,S)-HU211F was achieved from the commercially available (+)-(α)-Pinene (≥99% ee), that was first transformed into the corresponding (+)-(1*S*)-Myrtenol by means of an allylic oxidation promoted by SeO₂,⁹ and then converted into **2b**. In brief, the common intermediate **1** was condensed with **2a** or **2b** in the presence of BF₃·Et₂O at -20°C, affording the protected esters **10a** and **10b**. Finally, reduction with LiAlH₄ gave the desired cannabinoids (-)-(R,R)-HU210F and (+)-(S,S)-HU211F.



Scheme 3. Synthesis of (-)-(R,R)-HU210F and (+)-(S,S)-HU211F

Reagents and conditions: i) $\text{BF}_3\text{Et}_2\text{O}$, DCM, -20°C to rt, 2h (55%); ii) LiAlH_4 , THF, 0°C , 2h (50%).

Since the source of chirality in the syntheses are the two 4-hydroxy-myrtene pivalate enantiomers, **2a** and **2b**, the ee of the two final compounds reflected the ee of the commercially available starting materials ($\geq 95\%$ ee for **2a** and $\geq 98\%$ ee for **2b**). The enantiomeric purity of the cannabinoids mimics was confirmed by an analytical chiral HPLC analysis, subsequently (-)-(R,R)-HU210F and (+)-(S,S)-HU211F were purified in order to obtain pure ($>99\%$ e.e.) single enantiomers (see the experimental part for details).

Biological Tests

NMDAR antagonism

To assess the ability of (-)-(R,R)-HU210F and (+)-(S,S)-HU211F to antagonise NMDAR we made whole-cell patch-clamp recordings from primary rat cultured cortical neurons. Neurons were clamped at -60 mV and NMDA ($50\text{ }\mu\text{M}$) applied to evoke a current that was mediated predominantly by diheteromeric GluN1/GluN2B NMDAR (McKay et al., 2012)). Once a steady-state response was observed, we co-applied each compound of interest and assessed the percentage reduction in current (Fig. 3). To control for desensitisation of response during continued agonist exposure, we also applied NMDA with vehicle alone (1% DMSO) (Fig. 3A). We found that (-)-(R,R)-HU210F showed no NMDAR antagonism when compared to vehicle alone (Fig. 3B, F). In contrast, (+)-(S,S)-HU211F gave a maximal block of around 80% at the concentration used (Fig. 3C, F). In addition, we compared NMDAR antagonism of the parent compound, (+)-(S,S)-HU211, which we found blocked NMDAR-mediated current to an extent not significantly different from that seen with (+)-(S,S)-HU211F (Fig. 3D, F). We noted a slow blocking on-rate with both (+)-(S,S)-

HU211 and (+)-(S,S)-HU211F which was not due to slow rates of perfusion as MK-801 (a NMDAR open channel blocker which binds at a similar site) blocked NMDAR-mediated responses rapidly (Fig. 3E).

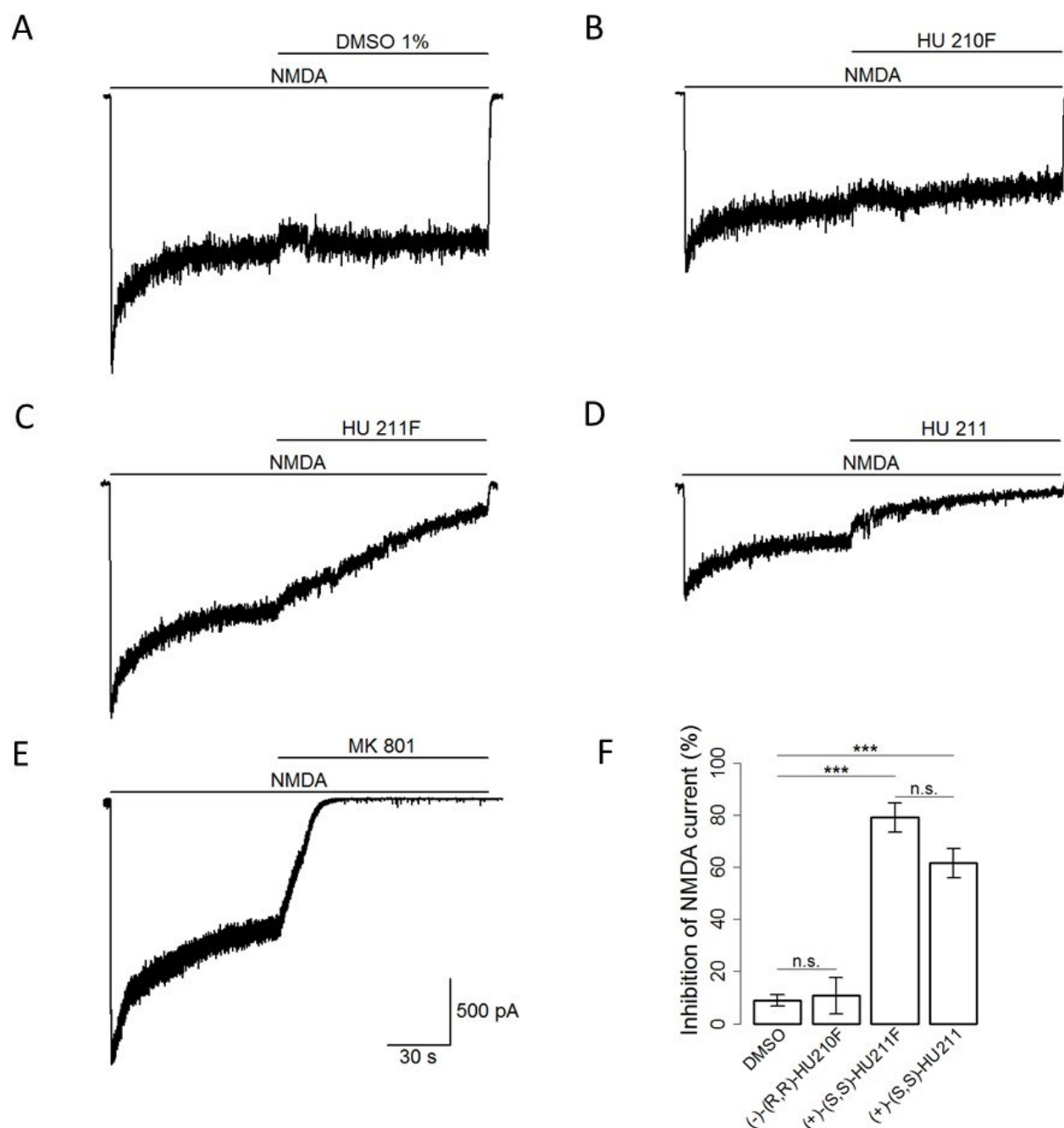


Figure 3: (+)-(S,S)-HU211F and (+)-(S,S)-HU211 are both NMDAR antagonists. (A-E) Example whole-cell current recordings from rat cortical neurons. **(A)** NMDA (50 μ M) with vehicle (1% DMSO), demonstrating desensitisation (mean percentage reduction in current compared to NMDA alone 8.7 ± 5.9 ($n=7$ cells)). **(B)** NMDA co-applied with (-)-(R,R)-HU210F (100 μ M), showing no additional blockade compared to vehicle (10.8 ± 17.5 (6), $p=0.995$). **(C)** NMDA co applied with (+)-(S,S)-HU211F (100 μ M), showing significant block compared to vehicle ($79.2 \pm 15.8\%$ (8), $p<0.0001$). **(D)** NMDA co applied with (+)-(S,S)-HU211 (100 μ M), showing significant block compared to vehicle ($61.6 \pm 13.9\%$ (6), $p<0.0001$) but not significantly different to (+)-(S,S)-HU211F ($p=0.115$). **(E)** NMDA co-applied with MK-801 (10 μ M), demonstrating faster on rate and 100% block. Scale bar applies to all traces. **(F)** Summary data. Data represent mean \pm SEM. Mean percentage inhibition of NMDA currents for vehicle alone and the three compounds of interest were first compared using a one way ANOVA, which showed a significant effect of compound ($F(3,23) = 46.9$, $p<1E-9$). We then compared individual means using post-hoc Tukey t-tests. *** $p<0.001$.

Conclusions

Our experiments assessing NMDAR antagonism confirmed that (+)-(S,S)-HU211 is a NMDAR antagonist and that its potency is retained in its fluorinated form (+)-(S,S)-HU211F. The finding that (+)-(S,S)-HU211 is an NMDAR antagonist is consistent with previous work using radioligands, behavioural assays, excitotoxicity assays and calcium influx (Feigenbaum 1989, Esshar 1993, Biegon and Joseph 1995). Based on the previously reported IC₅₀ of 11 μ M (Esshar 1993), we would have predicted a more complete block at the concentration used here (100 μ M). However, this IC₅₀ was based on radioligand studies assessing displacement of MK-801 whereas our work is the first electrophysiological assessment of the effect of (+)-(S,S)-HU211. Using electrophysiology to assess the effect of (+)-(S,S)-HU211 and its fluorinated analogue has also allowed us to highlight their very slow on-rate, a property not identified previously.

ExperimentalSection

2-(3,5-dimethoxyphenyl)-2-methylpropanenitrile (4)

To a stirred suspension of sodium hydride (60% in mineral oil, 677 mg, 16.9 mmol, 3eq) in dry DMF (5.0 mL) at 0°C was added dropwise a solution of the commercial available 2-(3,5-dimethoxyphenyl)acetonitrile **3** (1.0 g, 5.64 mmol, 1eq) and iodomethane (1.1 mL, 16.9 mmol, 3 eq) in dry DMF (5.0 mL). The reaction temperature rose to 25°C over a 15 min period and stirring was continued for 2 h. The reaction mixture was quenched with saturated aqueous NH₄Cl solution (5.0 mL) and diluted with diethyl ether (10 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The combined organic layer was washed with water and brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give compound **4** (1.1 g, 98%) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 8:2).

¹H NMR (400 MHz, CDCl₃) δ : 1.73 (s, 6H), 3.84 (s, 6H), 6.43 (t, 1 H, *J* = 2.2 Hz), 6.63 (d, 2H, *J* = 2.2 Hz). MS (ESI) for C₁₂H₁₅NO₂: *m/z* calculated 206.1 [M+H]⁺, 228.1 [M+Na]⁺; *m/z* found (relative intensity) 206.1 [M+H]⁺ (100), 228.1 [M+Na]⁺ (45).

2-(3,5-dimethoxyphenyl)-2-methylpropanal (5)

To a solution of **4** (1.21 g, 5.90mmol, 1eq) in dry DCM (50.0 mL) at -78°C was added DIBALH (1M solution in hexane, 14.75 mL, 14.75 mmol, 2.5 eq). The reaction mixture was stirred at the same temperature for 1 h and then quenched by dropwise addition of potassium sodium tartrate (10% solution in water, 20 mL). The resulting mixture was warmed to room temperature, stirred vigorously for 1 h, and then diluted with EtOAc (20 mL). The organic phase was separated and the aqueous phase extracted with EtOAc (3 x 50mL). The combined organic layer was washed with water and brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give aldehyde **5** (1.14 g, 93%) as a colorless oil.

R_f 0.37 (Hexane/EtOAc 8:2).

¹H NMR (400 MHz, CDCl₃) δ : 1.46 (s, 6H), 3.81 (s, 6H), 6.29-6.53 (m, 3 H), 9.49 (s, 1H).

¹³C NMR (100MHz, CDCl₃) δ : 22.6 (x 2 C), 50.6, 55.3 (x 2 C), 98.7, 105.2 (x 2 C), 143.7, 161.2 (x 2 C), 201.9.

MS (ESI) for C₁₂H₁₆O₃: *m/z* calculated 209.1 [M+H]⁺; *m/z* found (relative intensity) 209.1 [M+H]⁺ (100).

5-(bromotriphenyl- λ^5 -phosphanyl)pentan-1-ol (6)

A solution the commercially available 5-bromo-1-pentanol (2.0 mL, 16.6 mmol, 1 eq) in EtOH (35 mL) was added triphenylphosphine (4.35 g, 16.6 mmol, 1 eq) and K₂CO₃ (2.30 g, 16.6 mmol, 1 eq) and heated at reflux overnight. The solvent was evaporated under reduced pressure, the crude product was dissolved

in toluene (35 mL) and the mixture was stirred vigorously at 100 °C for 5 min. The mixture was cooled down and the phosphonium salt crystallized was collected by filtration. The resulting product **6** (9.6 g, 74%), obtained as with solid, was used crude, without further purification.

MS (ESI) for C₂₃H₂₅OP: m/z calculated 349.2 [M+H]⁺; m/z found (relative intensity) 349.1 [M+H]⁺ (100).

(5E)-7-(3,5-dimethoxyphenyl)-7-methyloct-5-en-1-ol (7)

To a suspension of phosphonium salt **6** (11.7 g, 27.3 mmol, 5 eq) in dry THF (130 mL) at 0 °C was added dropwise LiHMDS (1 M in THF, 27.3 mL, 27.3 mmol, 5 eq). The mixture was warmed to 10 °C and stirred for 30 min to ensure complete formation of the orange ylide. A solution of aldehyde **5** (1.1 g, 5.46 mmol, 1eq) in THF (15 mL) was added dropwise to the resulting slurry at the same temperature. The reaction was stirred overnight at room temperature. The mixture was quenched by the addition of saturated aqueous NH₄Cl (10 mL). The organic layer was separated and the aqueous phase was extracted with Et₂O (3 x 100). The combined organic layer was washed with brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 7:3) to give alkene **7** (1.4 g, 92%, single diastereoisomer) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 6:4).

¹H NMR (400 MHz, CDCl₃) δ : 1.18-1.26 (m, 2H), 1.32-1.40 (m, 2H), 1.41 (s, 6H), 1.68 (ddd, 2 H, J = 1.7, 7.4, 14.9 Hz), 3.46 (t, 2 H, J = 6.6 Hz), 3.80 (s, 6H), 5.29 (dt, 1H, J = 7.4, 11.4 Hz), 5.66 (dt, 1H, J = 1.7, 11.4 Hz), 6.31 (t, 1H, J = 2.3 Hz), 6.58 (d, 2H, J = 2.3 Hz).

¹³C NMR (100MHz, CDCl₃) δ : 25.2, 27.9, 31.3 (x 2 C), 32.0, 40.3, 55.3 (x 2 C), 62.7, 96.9, 105.0 (x 2 C), 131.0, 139.6, 153.3, 160.4 (x 2 C).

MS (ESI) for C₁₇H₂₆O₃: m/z calculated 279.2 [M+H]⁺, 301.2 [M+Na]⁺; m/z found (relative intensity) 279.2 [M+H]⁺ (100), 301.2 [M+Na]⁺ (60).

7-(3,5-dimethoxyphenyl)-7-methyloctan-1-ol (8)

To a solution of **7** (1.39 g, 5.0 mmol, 1 eq) in EtOAc (200 mL) was added 10% Pd/C (139 mg), and the resulting suspension was stirred vigorously under hydrogen atmosphere overnight at room temperature. The catalyst was removed by filtration through Celite and the filtrate was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 6:4) to give the saturated compound **8** (1.40 g, quantitative yield) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 6:4).

¹H NMR (400 MHz, CDCl₃) δ : 1.06-1.14 (m, 2H), 1.21-1.36 (m, 4H), 1.28 (s, 6H), 1.49-1.54 (m, 2H), 1.56-1.60 (m, 2H), 3.62 (t, 2 H, J = 6.6 Hz), 3.82 (s, 6H), 6.32 (t, 1H, J = 2.2 Hz), 6.51 (d, 2H, J = 2.2 Hz).

¹³C NMR (100MHz, CDCl₃) δ : 24.6, 25.6 (x 2 C), 29.0, 30.0, 32.8, 38.0, 44.4, 55.2 (x 2 C), 63.0, 96.6, 104.7 (x 2 C), 152.5, 160.5 (x 2 C).

MS (ESI) for C₁₇H₂₈O₃: m/z calculated 281.2 [M+H]⁺, 303.2 [M+Na]⁺; m/z found (relative intensity) 281.2 [M+H]⁺ (100), 303.2 [M+Na]⁺ (40).

5-(8-hydroxy-2-methyloctan-2-yl)benzene-1,3-diol (9)

To a solution of compound **8** (336mg, 1.20mmol, 1 eq) in dry Et₂O (5.0 mL) and dry THF (0.4 mL) MeMgI (3 M in Et₂O, 8.0 mL, 24.0 mmol, 20 eq) was added at 0 °C. After the mixture was heated to 100 °C in vacuum, the residue was then heated at 170 °C for 1 h under a flux of nitrogen. The cooled reaction mixture was quenched with saturated aqueous NH₄Cl (10 mL), and extracted with EtOAc (5 x 20 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and the solvent was evaporated under

reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 1:1) to give alcohol **9** (212mg, 70%) as a waxy white solid.

R_f 0.20 (Hexane/EtOAc 6:4).

^1H NMR (400 MHz, CD_3OD) δ : 1.06-1.14 (m, 2H), 1.18-1.34 (m, 4H), 1.23 (s, 6H), 1.43-1.51 (m, 2H), 1.55-1.59 (m, 2H), 3.52 (t, 2H, $J = 6.6$ Hz), 6.10 (t, 1H, $J = 2.2$ Hz), 6.31 (d, 2H, $J = 2.2$ Hz).

^{13}C NMR (100MHz, CD_3OD) δ : 24.4, 25.4, 28.1 (x 2 C), 29.9, 32.2, 37.1, 44.2, 61.6, 99.3, 104.4 (x 2 C), 151.9, 157.6 (x 2 C).

MS (ESI) for $\text{C}_{15}\text{H}_{24}\text{O}_3$: m/z calculated 253.2 $[\text{M}+\text{H}]^+$; m/z found (relative intensity) 253.2 $[\text{M}+\text{H}]^+$ (100).

5-(8-fluoro-2-methyloctan-2-yl)benzene-1,3-diol (1)

To a solution of alcohol **9** (140mg, 0.55mmol, 1.0 eq) in DCM (10 mL) at -78°C , DAST (0.1 mL, 0.66mmol, 1.2eq) is added dropwise. The reaction was stirred at 0°C for 15 min, then a saturated aqueous solution of NaHCO_3 (10 mL) was added. The resulting mixture was extracted with DCM (2 x 100 mL), dried over Na_2SO_4 , filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 6:4) to give fluorinated compound **1** (70mg, 50%) as waxy white solid.

R_f 0.35 (Hexane/EtOAc 6:4).

^1H NMR (400 MHz, CD_3OD) δ : 1.07-1.15 (m, 2H), 1.24 (s, 6H), 1.24-1.37 (m, 4H), 1.55-1.68 (m, 4H), 4.38 (dt, 2H, $J_{\text{H-H}} = 6.2$, $J_{\text{H-F}} = 47.6$ Hz), 6.10 (t, 1H, $J = 2.1$ Hz), 6.31 (d, 2H, $J = 2.1$ Hz).

^{19}F NMR (376.45 MHz, CD_3OD) δ : -219.9 (tt, 1F, $J = 24.7$, 47.6 Hz).

^{13}C NMR (100MHz, CD_3OD) δ : 24.3, 24.8 (d, $J_{\text{C-F}} = 5.4$ Hz), 28.1, (x 2 C), 29.6, 30.1 (d, $J_{\text{C-F}} = 19.5$ Hz), 37.1, 44.1, 83.4, (d, $J_{\text{C-F}} = 163.7$ Hz), 99.3, 104.3, 151.9, 157.7 (x 2 C).

MS (ESI) for $\text{C}_{15}\text{H}_{23}\text{FO}_2$: m/z calculated 255.2 $[\text{M}+\text{H}]^+$, 277.2 $[\text{M}+\text{Na}]^+$; m/z found (relative intensity) 255.3 $[\text{M}+\text{H}]^+$ (100), 277.2 $[\text{M}+\text{Na}]^+$ (60).

[(6aR,10aR)-3-(8-fluoro-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[*c*]isochromen-9-yl]methyl 2,2-dimethylpropanoate (10a)

To a solution of resorcinol **1** (49 mg, 0.19 mmol, 1 eq) and pivalate ester **2a** (48 mg, 0.19 mmol, 1 eq) in dry DCM (65 mL) at -20°C was added borontrifluoride etherate (0.12 mL, 1.0 mmol, 5.3 eq). The mixture was allowed to warm up to room temperature and then stirred for 2 h. The mixture was carefully washed with brine, over Na_2SO_4 , filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 9:1) to give compound **10a** (51mg, 55%) as a waxy white solid.

R_f 0.60 (Hexane/EtOAc 8:2).

$[\alpha]_{\text{D}}^{27}$: -112 ($c = 0.8$, CHCl_3).

^1H NMR (400 MHz, CDCl_3) δ : 1.08-1.15 (m, 2H), 1.15 (s, 3H), 1.23 (s, 6H), 1.23-1.28 (m, 2H), 1.25 (s, 9H), 1.32-1.38 (m, 2H), 1.42 (s, 3H), 1.51-1.56 (m, 2H), 1.57-1.70 (m, 2H), 1.83-1.96 (m, 3H), 2.20-2.32 (m, 1H), 2.63-2.80 (m, 1H), 3.38 (dd, 1H, $J = 3.2$, 16.6 Hz), 4.42 (dt, 2H, $J_{\text{H-H}} = 6.2$, $J_{\text{H-F}} = 47.4$ Hz), 4.53 (d, 1H, $J = 4.1$ Hz), 4.75 (bs, OH), 5.78 (d, 1H, $J = 4.5$ Hz), 6.25 (d, 1H, $J = 1.6$ Hz), 6.41 (d, 1H, $J = 1.6$ Hz).

^{19}F NMR (376.45 MHz, CDCl_3) δ : -218.9 (tt, 1F, $J = 25.0$, 47.4 Hz).

^{13}C NMR (100MHz, CDCl_3) δ : 18.5, 24.5, 25.0 (d, $J_{\text{C-F}} = 5.5$ Hz), 27.3, (x 4 C), 27.6, 27.7, 28.7 (x 2 C), 29.8, 30.4 (d, $J_{\text{C-F}} = 19.3$ Hz), 31.2, 31.6, 37.3, 38.9, 44.3, 44.8, 68.0, 82.2, (d, $J_{\text{C-F}} = 163.9$ Hz), 105.4, 108.0, 109.8, 123.3, 134.0, 150.0, 154.5, 154.6, 178.5.

MS (ESI) for $\text{C}_{30}\text{H}_{45}\text{FO}_4$: m/z calculated 489.3 $[\text{M}+\text{H}]^+$; m/z found (relative intensity) 489.3 $[\text{M}+\text{H}]^+$ (100).

(-)-(R,R)-HU210F

A solution of the protected ester **10a** (47.9mg, 0.098mmol, 1 eq) in dry THF (1.0 ml) was added dropwise to a suspension of LiAlH_4 (15.3mg, 0.39mmol, 4 eq) in THF (1.0 ml) at 0 °C. The reaction mixture was stirred for 2 h and allowed to warm to room temperature. The reaction was quenched with water (2 mL) and extracted with ether (2 x 5 mL).

The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 8:2) to give the final compound **(-)-(R,R)-HU210F** (19.8mg, 50%) as a hygroscopic white solid.

R_f 0.30 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: -128 (c = 0.6, CHCl_3).

^1H NMR (400 MHz, CDCl_3) δ : 1.07-1.14 (m, 2 H), 1.12 (s, 3H), 1.22 (s, 6 H), 1.22-1.28 (m, 2 H), 1.31-1.37 (m, 2 H), 1.41 (s, 3 H), 1.50-1.54 (m, 2 H), 1.58-1.71 (m, 2 H), 1.82-1.93 (m, 3H), 2.25 (dd, 1H, J = 4.4, 11.0 Hz), 2.73 (td, 1H, J = 4.6, 10.9 Hz), 3.47 (dd, 1H, J = 4.0, 15.7 Hz), 4.09 (q, 2H, J = 12.7 Hz), 4.42 (dt, 2H, $J_{\text{H-H}}$ = 6.2, $J_{\text{H-F}}$ = 47.4 Hz), 5.54 (bs, OH), 5.77 (d, 1H, J = 4.7 Hz), 6.25 (d, 1H, J = 1.8 Hz), 6.40 (d, 1H, J = 1.8 Hz).

^{19}F NMR (376.45 MHz, CDCl_3) δ : -217.8 (tt, 1F, J = 25.0, 47.4 Hz).

^{13}C NMR (100MHz, CDCl_3) δ : 18.5, 24.5, 25.0 (d, $J_{\text{C-F}}$ = 5.5 Hz), 27.6, 27.7, 28.7 (x 2 C), 29.9, 30.4 (d, $J_{\text{C-F}}$ = 19.3 Hz), 31.3, 31.5, 37.3, 44.2, 45.0, 67.1, 76.5, 84.3, (d, $J_{\text{C-F}}$ = 163.8 Hz), 105.5, 107.8, 109.9, 121.5, 138.2, 149.9, 154.5, 154.7.

MS (ESI) for $\text{C}_{25}\text{H}_{37}\text{FO}_3$: m/z calculated 405.3 $[\text{M}+\text{H}]^+$; m/z found (relative intensity) 405.3 $[\text{M}+\text{H}]^+$ (100). HRMS calcd. for $\text{C}_{25}\text{H}_{38}\text{F}_1\text{O}_3$: 405.2799, found: 405.2800.

[(6aS,10aS)-3-(8-fluoro-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-9-yl]methyl 2,2-dimethylpropanoate (10b)¹

To a solution of resorcinol **1** (42 mg, 0.16 mmol, 1 eq) and pivalate ester **2b** (41 mg, 0.16 mmol, 1 eq) in dry DCM (60 mL) at -20 °C was added borontrifluoride etherate (0.10 mL, 0.85 mmol, 5.3 eq). The mixture was allowed to warm up to room temperature and then stirred for 2 h. The mixture was carefully washed with brine, over Na_2SO_4 , filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 9:1) to give compound **10b** (43mg, 55%) as a waxy white solid.

R_f 0.60 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: +112 (c = 0.8, CHCl_3).

^1H NMR (400 MHz, CDCl_3) δ : 1.07-1.17 (m, 2 H), 1.14 (s, 3H), 1.23 (s, 6 H), 1.23-1.28 (m, 2 H), 1.25 (s, 9 H), 1.31-1.38 (m, 2 H), 1.42 (s, 3 H), 1.51-1.55 (m, 2 H), 1.58-1.71 (m, 2 H), 1.83-1.95 (m, 3H), 2.25-2.39 (m, 1 H), 2.70-2.76 (m, 1 H), 3.38 (dd, 1H, J = 3.2, 16.6 Hz), 4.42 (dt, 2H, $J_{\text{H-H}}$ = 6.2, $J_{\text{H-F}}$ = 47.4 Hz), 4.53 (d, 1H, J = 4.1 Hz), 4.89 (bs, OH), 5.78 (d, 1H, J = 4.5 Hz), 6.25 (d, 1H, J = 1.6 Hz), 6.41 (d, 1H, J = 1.6 Hz).

^{19}F NMR (376.45 MHz, CDCl_3) δ : -218.9 (tt, 1F, J = 25.0, 47.4 Hz).

^{13}C NMR (100MHz, CDCl_3) δ : 18.5, 24.5, 25.0 (d, $J_{\text{C-F}}$ = 5.5 Hz), 27.3, (x 4 C), 27.6, 27.7, 28.7 (x 2 C), 29.8, 30.4 (d, $J_{\text{C-F}}$ = 19.3 Hz), 31.2, 31.6, 37.3, 38.9, 44.3, 44.8, 68.0, 82.2, (d, $J_{\text{C-F}}$ = 163.9 Hz), 105.4, 108.0, 109.8, 123.3, 134.0, 150.0, 154.5, 154.6, 178.5.

MS (ESI) for $\text{C}_{30}\text{H}_{45}\text{FO}_4$: m/z calculated 489.3 $[\text{M}+\text{H}]^+$; m/z found (relative intensity) 489.3 $[\text{M}+\text{H}]^+$ (100).

(+)-(S,S)-HU211F¹

A solution of the protected ester **10b** (40.0mg, 0.082mmol, 1 eq) in dry THF (1.0 ml) was added dropwise to a suspension of LiAlH_4 (12.4mg, 0.33mmol, 4 eq) in THF (1.0 ml) at 0 °C. The reaction mixture was stirred for 2 h and allowed to warm to room temperature. The reaction was quenched with water (2 mL) and extracted with ether (2 x 5 mL).

The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 8:2) to give the final compound (+)-(S,S)-**HU211F** (16.6mg, 50%) as a hygroscopic white solid.

^1H NMR (400 MHz, CDCl_3) δ : 1.08-1.15 (m, 2 H), 1.12 (s, 3H), 1.22 (s, 6 H), 1.22-1.31 (m, 2 H), 1.31-1.37 (m, 2 H), 1.41 (s, 3 H), 1.50-1.54 (m, 2 H), 1.58-1.71 (m, 2 H), 1.85-1.94 (m, 3H), 2.26 (dd, 1H, $J = 4.4, 11.0$ Hz), 2.73 (td, 1H, $J = 4.6, 10.9$ Hz), 3.45 (dd, 1H, $J = 4.0, 15.7$ Hz), 3.92 (bs, OH), 4.12 (q, 2H, $J = 12.7$ Hz), 4.42 (dt, 2H, $J_{\text{H-H}} = 6.2, J_{\text{H-F}} = 47.4$ Hz), 5.77 (d, 1H, $J = 4.7$ Hz), 6.25 (d, 1H, $J = 1.8$ Hz), 6.40 (d, 1H, $J = 1.8$ Hz).

^{19}F NMR (376.45 MHz, CDCl_3) δ : -217.8 (tt, 1F, $J = 25.0, 47.4$ Hz).

^{13}C NMR (100MHz, CDCl_3) δ : 18.5, 24.5, 25.0 (d, $J_{\text{C-F}} = 5.5$ Hz), 27.6, 27.7, 28.7 (x 2 C), 29.9, 30.4 (d, $J_{\text{C-F}} = 19.3$ Hz), 31.3, 31.5, 37.3, 44.2, 45.0, 67.1, 76.5, 84.3, (d, $J_{\text{C-F}} = 163.8$ Hz), 105.5, 107.8, 109.9, 121.5, 138.2, 149.9, 154.5, 154.7.

R_f 0.30 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: +128 ($c = 0.6$, CHCl_3).

HRMS calcd. for $\text{C}_{25}\text{H}_{38}\text{F}_1\text{O}_3$: 405.2799, found: 405.2800.

Culturing of primary rat cortical neurons

Culturing was a modified version of (Furshpan and Potter, 1989). Brains from E20.5 Sprague Dawley rats (sex not determined) were microdissected in medium containing (in mM): Na_2SO_4 81.8, K_2SO_4 30, MgCl_2 5.84, CaCl_2 0.252, HEPES 1, Phenol Red 0.001%, 2 D-glucose 20, kynurenic acid 1. Cortices were incubated for 40 minutes in papain enzyme (10 U / ml) (Worthington Biochemical Corporation, Lakewood, NJ, USA) and L-cysteine (3.7 mM) then washed and triturated in NeuroBasal A medium (supplemented with 1% rat serum (Harlan laboratories, Indianapolis, IN, USA), 1 x B-27 supplement, 1% antibacterial/antimycotic and 1 mM glutamine). The cell suspension was diluted in opti-MEM (supplemented with 20 mM glucose and 1% antibacterial/antimycotic) to give an end concentration of 1 hemisphere per 14 ml, and 0.5 ml/coverslip plated onto 13mm diameter coverslips precoated with poly-D-lysine (1.33% w/v in H_2O) and laminin (0.5% w/v) (Roche, Basel, Switzerland) in 24 well plates. Plates were kept at 37°C in a humidified 5% CO_2 incubator for 2.5 hours before replacement of the cell suspension with supplemented NeuroBasal A. On the fourth day *in vitro* (DIV 4) 1 ml/well of supplemented NeuroBasal A containing 9.6 μM cytosine β -D-arabinofuranoside hydrochloride was added to the cells.

Whole cell voltage clamp recordings in neurons

Recordings were made on DIV7 at room temperature (18–21 °C) with neurons superfused (at a flow rate of 2 mL/min) with an external recording solution comprising (in mM) NaCl 150, KCl 2.8, HEPES 10, CaCl_2 0.5, glucose 10, glycine 0.05, diethylene triaminepentaacetic acid 0.01, tetrodotoxin 0.0003; pH 7.35 using NaOH (300–330 mOsm). 50 μM NMDA was applied alone for 80 s (to allow response to stabilise), then 50 μM NMDA was applied along with the compound of interest or vehicle alone for 110 s (lengthy application required due to compound's slow rate of block). The average current during the final 10 s of response to NMDA alone or NMDA plus compound was used to calculate percentage inhibition. 100 μM of (-)-(R,R)-HU210F, (+)-(S,S)-HU211F and (+)-(S,S)-HU211 were used, 10 μM of MK-801 and vehicle was 1% dimethyl sulfoxide. Application of solutions was controlled manually. Patch-pipettes were made from thick-walled borosilicate glass (GC150F-7.5, Harvard Apparatus) using a P-87 puller (Sutter Instruments)

to give a resistance of 2–4 MΩ when filled with an internal solution containing (in mM): K–gluconate 141, NaCl 2.5, HEPES 10, EGTA 11; pH 7.3 with KOH (300 mOsm). Currents were recorded using an Axopatch 200B amplifier (Molecular Devices). Data were filtered at 5 kHz and digitized at 20 kHz via a National Instruments BNC–2090A analogue–digital interface (National Instruments, Newbury, UK) using WinEDR software (Strathclyde Electrophysiology Software). Neurons were voltage–clamped at –60 mV, and recordings were rejected if the holding current was greater than 150 pA or if the series resistance was greater than 30 MΩ.

References

- ¹R. Mechoulam, N. Lander, A. University and J. Zahalka, *Tetrahedron: Asymmetry*, 1990, **1**, 315–318.
 - ²J. Liddle and J. W. Huffman, *Tetrahedron*, 2001, **57**, 7607–7612.
 - ³R. Sharma, S. P. Nikas, C. A. Paronis, J. T. Wood, A. Halikhedkar, J. J. Guo, G. A. Thakur, S. Kulkarni, O. Benchama, J. G. Raghav, R. S. Gifford, T. U. C. Järbe, J. Bergman and A. Makriyannis, *J. Med. Chem.*, 2013, **56**, 10142–10157.
 - ⁴D. P. Papahatjis, V. R. Nahmias, S. P. Nikas, T. Andreou, S. O. Alapafuja, A. Tsotinis, J. Guo, P. Fan and A. Makriyannis, *Journal of Medicinal Chemistry*, 2007, **50**, 4048–4060.
 - ⁵(a) A. I. Meyers and E. W. Collington, *Tetrahedron*, 1971, **27**, 5979–5985; (b) H. Lei and J. Atkinson, *J. Org. Chem.*, 2000, **65**, 2560–2567.
 - ⁶D. P. Papahatjis, T. Kourouli, V. Abadji, A. Goutopoulos and A. Makriyannis, *Journal of medicinal chemistry*, 1998, **41**, 1195–1200.
 - ⁷(a) W. Lytollis, R. T. Scannell, H. An, V. S. Murty, K. S. Reddy, J. R. Barr and S. M. Hecht, *Journal of the American Chemical Society*, 1995, **117**, 12683–12690; (b) T. R. Hoyer, P. E. Humpal and B. Moon, *J. Am. Chem. Soc.*, 2000, **122**, 4982–4983.; (c) A. Tanaka, Y. Arai, S.-N. Kim, J. Ham and T. Usuki, *Journal of Asian Natural Products Research*, 2011, **13**, 290–296.
 - ⁸R. P. Singh and J. M. Shreeve, *Synthesis*, 2002, 2561–2578.
 - ⁹I. A. Dvornikova, L. L. Frolova, A. V. Kuchin and I. P. Beletskaya, *Russian Journal of Organic Chemistry*, 2007, **43**, 352–358.
- Eshhar N, Striem S, Biegon A. *Neuroreport*. 1993 Dec 13;5(3):237-40. HU-211, a non-psychotropic cannabinoid, rescues cortical neurones from excitatory amino acid toxicity in culture.
- Biegon A, Joseph AB. *Neurol Res*. 1995 Aug;17(4):275-80. Development of HU-211 as a neuroprotectant for ischemic brain damage.
- J JFeigenbaum, F Bergmann, S A Richmond, R Mechoulam, V Nadler, Y Kloog, and M Sokolovsky. *Proc Natl AcadSci U S A*. 1989 Dec; 86(23): 9584–9587. Nonpsychotropic cannabinoid acts as a functional N-methyl-D-aspartate receptor blocker.
- S McKay,* NH Griffiths,* PA Butters,* EB Thubron, GE Hardingham, and DJA Wyllie. *Br J Pharmacol*. 2012 Jun; 166(3): 924–937. Direct pharmacological monitoring of the developmental switch in NMDA receptor subunit composition using TCN 213, a GluN2A-selective, glycine-dependent antagonist
- Furshpan EJ, Potter DD. *Neuron*. 1989 Aug;3(2):199-207. [Seizure-like activity and cellular damage in rat hippocampal neurons in cell culture.](#)